IVD

For professional use only

HBV Quantitative Real-Time PCR Kit

(*PREP-NA* DNA/RNA Extraction Kit included)

USER MANUAL

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1. INTENDED USE

The **HBV Quantitative Real-Time PCR Kit** is intended for research and diagnostic applications as well as for evaluation of the therapy efficacy. The **HBV Quantitative Real-Time PCR Kit** is an *in vitro* Nucleic Acid Test (NAT) – based pathogen detection and quantification product. The **HBV Quantitative Real-Time PCR Kit** is designed to detect and quantitate Hepatitis B Virus (HBV) nucleic acids in human blood plasma samples with an aid of Quantitative Real-Time Polymerase Chain Reaction (qPCR) method.

The **HBV Quantitative Real-Time PCR Kit** can be used in clinical practice for HBV diagnostics.

2. METHOD

The implemented PCR method is based on amplification of a target DNA sequence.

The **HBV Quantitative Real-Time PCR Kit** is based on real-time detection of the target DNA sequence.

Real-time PCR technology is based on measurement of the fluorescence at every cycle of reaction. The PCR-mix contains target-specific hydrolyzing probes bearing reporter and quencher molecules. Once hybridized to a target sequence, the probe become activated. As a result of activation fluorescence increases proportionally to target sequence amplification. The intensity of fluorescence is detected with a real-time PCR thermal cycler data collection unit and analyzed with the software provided.

The assay includes following steps:

-**DNA extraction**. On this step the internal control sample (IC) is added to the samples. It is needed for test quality assurance.

-**Real-time PCR amplification**. The Kit has passed validation on "DNA-Technology" made instruments and software REF O-DTPRIME4M1-EU, O-DTPRIME5M1-EU, O-DTLITE4S1-EU, O-DTLITE5S1-EU.

The **HBV Quantitative Real-Time PCR Kits** REF Q2-P602-23/9EU, Q2-P602-S3/9EU and Q2-P602-24/9EU are also approved for use with iQ5 (Bio-Rad Laboratories) and Rotor-Gene (Qiagen) thermal cyclers respectively. The Kit can be supplied in either separate (1x96) or stripped (8x12) tubes. (REF Q2-P602-23/9EU, Q2-P602-24/9EU and Q2-P602-S3/9EU respectively).

-**Quantitative analysis**. The quantitation of the target DNA is performed with an aid of Standards (ST) with known concentration of artificially synthesized target DNA. The Kit supplied with STs of the two concentrations $1x10^6$ (ST1) and $3x10^3$ copies/ml (ST2). The STs are used to build the standard curve which is necessary to quantitate the DNA in the sample.

3. CONTENT

Table 1. *PREP-NA* DNA/RNA Extraction Kit

Reagent	Description	Total volume	Amount
Lysis buffer	Colorless, soapy liquid	30 ml	1 vial
Precipitation buffer	Colorless liquid	40 ml	1 vial
Washout solution 1	Colorless liquid	50 ml	1 vial
Washout solution 2	Colorless liquid	30 ml	1 vial
Dissolving buffer	Colorless liquid	5 ml (1.25 ml in each tube)	4 tubes
Negative control (C-)	Colorless liquid	3 ml (1.5 ml in each tube)	2 tubes
Internal control (IC)	Colorless liquid	1 ml	1 tube

Table 2. Standards

Reagent	Description	Total volume	Amount
ST1 ($1x10^6$ copies/ml)	Colorless liquid	75 µL	1 tube
ST2 ($3x10^3$ copies/ml)	Colorless liquid	75 µL	1 tube

Table 3. HBV Quantitative Real-Time PCR Kit

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Composition of colorless liquid and white waxy fractions	1.92 ml (0.02 µL per tube)	96 separate (1x96) or stripped (8x12) tubes
TECHNO Taq-polymerase	Colorless viscous liquid	50 µL	1 tube
PCR-buffer	Colorless liquid	1 ml (0.5 ml in each tube)	2 tubes
Positive control (C+)	Colorless liquid	150 µL	1 tube
Mineral oil (not supplied in Kit for Rotor-Gene)	Colorless viscous liquid	2 ml (1 ml in each tube)	2 tubes

The approximate total time needed to perform the assay is 4 hours

The *PREP-NA* DNA/RNA Extraction Kit is sufficient for extraction of 100 samples.

The **HBV Quantitative Real-Time PCR Kit** sufficient to test 44 samples in duplicates.

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1 **Specimen collection**

The whole blood samples shoud be collected in 2 or 4 ml Vacuette type tubes with EDTA in 2.0 mg/ml final concentration. The sodium citrate anticoagulant is also applicable.

The use of heparin anticoagulant is not allowed.

4.2 **DNA extraction and PCR**

Vortex mixer

Vacuum pump with collector to remove the supernatants

1.5 ml tubes

PCR tube rack for 0.2, and 1.5 ml tubes

Single channel pipettes (volume range 0.5-10 µL, 5-40 µL, 40-200 µL, 100-1000 µL)

RNase and DNase free filtered pipette tips (volume range 20 µL, 50 µL, 200 µL, 1000 µL)

Powder-free surgical gloves

Disinfectant solution

Container for used pipette tips

High speed centrifuge (13000 rpm)

Thermostat (temperature range 50-65°C)

Refrigerator

Real-time PCR thermal cycler

5. WARNINGS AND PRECAUTIONS

The laboratory makeup should comply the requirements regulating work with microorganisms of I-IV classes of pathogenicity.

Handle and dispose all biological samples, reagents and materials used to carry out the assay as if they were able to transmit infective agents. Avoid direct contact with the biological samples reagents and materials used to carry out the assay. Any material coming in contact with the biological samples must be treated for at least 30 minutes with disinfecting solution or autoclaved for 1 hour at 121ºC before disposal.

Molecular biology procedures, such as nucleic acids extraction, reverse transcription, amplification and detection require qualified staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.

All oligonucleotide components are produced by artificial synthesis technology according to internal quality control protocol and do not contain blood or products of blood processing.

Positive control is produced by artificial DNA synthesis technology. Positive control does not include parts of infectious agents.

All the liquid solutions are designed for single use and can not be used more than once in amplification reactions. Plastic tubes do not contain phthalates. Do not breathe gas/fumes/vapour/spray produced by the components of the kit. Do not eat/drink components of the kit. Avoid contact with eyes. Do not use the kit after the expiry date provided. Only use the reagents provided in the kit and those recommended by manufacturer. Do not mix reagents from different batches. Do not use reagents from third party manufacturers' kits.

Significant health effects are **NOT** anticipated from routine use of this kit when adhering to the instructions listed in the current manual.

6. DNA EXTRACTION PROTOCOL

The **HBV Quantitative Real-Time PCR Kit** is designed to detect DNA extracted from whole blood. Shake the tube containing blood sample thoroughly to mix the blood and anticoagulant.

The overall storage of the sample should not exceed 6 hours.

The transportation and storage temperature from collecting the sample till analysis should be in 2-4°C range.

6.1 To obtain the plasma spin the tubes with blood at 3000 rpm for 20 min at room temperature (18–25°C).

6.2 Take the upper fraction (plasma) with an automatic sampler and put it into the new 1.5 ml tube. The blood plasma can be stored at -20°C for 3 months.

The lysis buffer can contain the precipitate. Dissolve it at 65°C for 10 min. prior to use.

At this step of assay use only RNase and DNase free pipette tips.

To rise the reliability of the results it is advised to perform the extraction in duplicates.

6.3 Mark the required number of 1,5 ml tubes by the following scheme:

- 2 tubes for each sample to be tested
- 1 tube for the negative control (C-)
- 1 tube for the positive control (C+)

For example if you need to test 10 samples, mark 22 tubes (20 for the samples, 1 for C- and 1 for C+).

6.4 Add 10 µL of the premixed IC in each tube.

6.5 Add 300 µL of the lysis buffer avoiding contact of the pipette tip with an edge of the tube. Close the tubes.

Open the tube, add sample, then close the tube before proceeding to the next DNA sample to prevent contamination.

6.6 Add 100 µL of the blood plasma sample into the marked tubes. Do not add samples to the C- and C+ tubes.

6.7 Add 100 µL of the C- and C+ into corresponding tubes.

6.8 Close the tubes and mix them for 3–5 s twice.

6.9 Incubate the tubes for 15 min at 65°C, spin down the drops at 13000 rpm for 30 s at room temperature (18–25°C).

6.10 Add 400 µL of the precipitation buffer into all tubes. Close the tubes and mix them for 3–5 s. twice.

6.11 Spin the tubes at 13000 rpm for 15 min at room temperature (18–25°C).

6.12 Remove the supernatant avoiding contact of the pipette tip with the precipitate. Use new tip for each sample.

6.13 Add 500 µL of the washout solution №1 to the precipitate and shake the tube thoroughly

6.14 Spin the tubes at 13000 rpm for 5 min at room temperature (18–25°C).

6.15 Remove the supernatant avoiding contact of the pipette tip with the precipitate. Use new tip for each sample.

6.16 Add 300 µL of the washout solution №2 to the precipitate and shake the tube thoroughly.

6.17 Spin the tubes at 13000 rpm for 5 min at room temperature (18–25°C).

6.18 Remove the supernatant avoiding contact of the pipette tip with the precipitate. Use new tip for each sample.

6.19 Open the tubes and dry the precipitate at 65°C for 5 min.

6.20 Add 25 µL of the dissolving buffer to the precipitate. Spin down the drops for 3–5 s.

6.21 Incubate the tubes for 10 min at 65°C.

6.22 Spin down the drops at 13000 rpm for 30 s.

The nucleic acid preparation is ready.

The preparation can be stored at -20°C for 1 month or at -70 °C for 1 year.

7. PCR PROTOCOL

7.1 Mark tubes with PCR-mix for each test sample, negative control (C-), positive control (C+) and three tubes for each of the Standards (ST1 and ST2).

For example if you need to test 10 samples, mark 28 tubes (20 for each sample in duplicate, 1 for C-, 1 for C+, 3 for ST1 and 3 for ST2).

Mark only the caps of the tubes when using Rotor-Gene Thermal Cycler.

7.2 Thaw PCR-buffer at the room temperature

7.3 Mix the PCR-buffer and TECHNO Taq-polymerase thoroughly (3-5 sec), then spin briefly (1-3 sec) at room temperature (18–25°C).

Hold TECHNO Taq-polymerase at room temperature as short time as possible. The overheating is detrimental to its performance.

7.4 Prepare the mixture of PCR-buffer and TECHNO Taq-polymerase (TECHNO Taq-polymerase solution). Add into the one tube:

10 x (N+1) µL of PCR-buffer,

0,5 × (N+1) µL of TECHNO Taq-polymerase,

N — number of the marked tubes including C-, C+, ST1 and ST2

7.5 Vortex the tube with TECHNO Taq-polymerase solution for 3-5 seconds and spin down the drops for 1-3 seconds at room temperature (18–25°C). The maximum storage time for TECHNO Taq-polymerase solution is 1 hour.

7.6 Add 10 µL of TECHNO Taq-polymerase solution into each tube. Avoid paraffin layer break.

7.7 Add 20 µL of mineral oil into each tube. Avoid paraffin layer break (skip this step when using Q2-P602-24⁄9EU). Close the tubes.

7.8 Vortex the tubes with samples for 3-5 seconds and spin down the drops for 1-3 seconds.

7.9 Add 5,0 µL of DNA sample into corresponding tube. Avoid paraffin layer break.

Open the tube, add DNA sample, then close the tube before proceeding to the next DNA sample to prevent contamination. Use filter tips.

7.10 Add 5,0 µL of C-, C+, ST1 and ST2 into corresponding tubes. Avoid paraffin layer break.

7.11 Spin tubes briefly (1-3 sec).

7.12 Set the tubes to Real-Time PCR Termal Cycler.

7.13 Launch the Thermal Cycler software and run PCR according to instructions supplied with device. See table 4-7 to refer the cycling program an table 8 to refer the detection channels.

Table 4. The PCR program for DT*lite* and DT*prime* Thermal Cyclers

Step	Temperature, °C	Min.	Sec.	Number of cycles	Optical measurement	Type of the step
1	94	5	00	1		Cycle
2	94	0	10	50		Cycle
	62	0	20		v	
5	10	…	…	…		Holding

Table 5. The PCR program for iCycler iQ5 thermal cyclers (with persistent well factor)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
1	1				
		1	5 min	94	
2	50				
		1	10 sec	94	
		2	20 sec	62	Real Time
3	1				
4	…	…		10	Storage

Table 6. The PCR program for iCycler iQ5 thermal cyclers (with dynamic factor)

Cycle	Repeats	Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
dynamicwf.tmo program					
1	1				
		1	00:30	80,0	
		2	05:00	94,0	
2	5				
		1	00:20	94,0	
		2	00:30	62,0	
3	2				
		1	00:20	80,0	Real Time
PCR program					
4	45				
		1	00:10	94,0	
		2	00:20	62,0	Real Time
5		…	…	10,0	Storage

Table 7. The PCR program for Rotor-Gene Thermal Cyclers

Cycling	Temperature	Hold Time	Cycle Repeats
Cycling	80 °C	300 sec	1 time
Cycling 2	94 °C	10 sec	50 times
	58 °C	5 sec	
	62 °C*	25 sec	

* Take the measurement

Table 8. Detection channels

	Specific product	IC
DT*prime*, DT*lite* and IQ5	FAM	HEX
Rotor-Gene	Green	Yellow

8. CONTROLS

Table 9

Control	The controlled step	Result		Interpretation
		Specific signal is present	Specific signal is absent	
C+	PCR	+	+	Valid
		-	-	Invalid
C-	PCR and DNA extraction	+	+	Invalid
		-	-	Valid
IC	PCR DNA extraction	+	+	Valid
		-		Valid
			-	Invalid

The sample is considered positive if the signal for specific DNA is present. The signal for IC could be absent in samples with high concentration of specific DNA due to competitive priming.

The sample is considered negative if the signal for specific DNA is absent and for IC is present.

If the signal for C- is present, whole tests of current batch considered false. Decontamination required.

9. DATA ANALYSIS

The analysis performed automatically. After completion of the run the device will build standard curve, define the concentration of viral DNA and form the report. The PCR efficiency should be in 90-100% range.
The interpretation should be performed in accordance with table 10.
Table 10

Detection channel		**Interpretation**
Fam/Green copies/ml	**Hex/Yellow** Cp (Ct)	
Test samples		
$7{,}5\times10^2$ – $1{,}0\times10^8$	Not considered	Positive with specified viral load (copies/ml)
Less than **$7{,}5\times10^2$**	Not considered	Positive with notification «**Less than 750 copies/ml**» (no specified value)
More than **$1{,}0\times10^8$**	Not considered	Positive with notification «**More than $1{,}0\times10^8$ copies/ml**» (no specified value)
Not specified (N/A)	Specified (Cp/Ct 29-34)	Negative
Not specified (N/A)	Not specified (N/A)	Uncertain
C+		
$2{,}2\times10^4 – 7{,}1\times10^4$ *	Not considered	Positive with specified viral load (copies/ml)
C-		
Not specified	Specified (Cp/Ct 29-34)	Negative

*If the concentration of the C+ falls out the $2{,}2\times10^4–7{,}1\times10^4$ range the test should be repeated

10 TROUBLESHOOTING

Table 11

	Specific signal +	Specific signal -	Possible cause	Solution
C+	-	-	Operation error PCR inhibition Violation of storage and handling requirements	Repeat whole test Dispose current batch
C-	+	+	Contamination	Dispose current batch Perform decontamination procedures
IC		-	PCR inhibition	Repeat whole test

11 STORAGE AND HANDLING REQUIREMENTS

Expiry date – 6 month from the date of production.

All components of the **HBV Quantitative Real-Time PCR Kit** (except PCR-mix, ST1, ST2 and C+) must be stored at -20°C over the storage period. The PCR-buffer and mineral oil can be stored at at 2-8°C.

The PCR-mix, ST1, ST2, C+ and *PREP-NA* DNA/RNA Extraction Kit must be stored at 2-8°C over the storage period.

Transportation can be held by all types of roofed transport with adherence to above mentioned temperature requirements.

An expired **HBV Quantitative Real-Time PCR Kit** must not be used.

We strongly recommend following the instructions to get robust and reliable results.

The conformity of the **HBV Quantitative Real-Time PCR Kit** to the prescribed technical requirements is subject to compliance of storage, carriage and handling conditions recommended by manufacturer.

Contact our customer service by quality issues of the **HBV Quantitative Real-Time PCR Kit**: 115587, Moscow, Varshavskoye sh. 125g building 6, DNA Technology, LLC. Phone/Fax: +7(495)9804555 e-mail: help@dna-technology.ru, www.dna-technology.ru

12 SPECIFICATIONS

a. **Analytical specificity:** the **HBV Quantitative Real-Time PCR Kit** allows detection of all known HBV subtypes. The samples containing HBV will be defined as positive and characterized quantitatively. The samples not containing HBV will be defined as negative.

b. **Linear range:** $7{,}5x10^2 – 1x10^8$ copies/ml

c. **Variation coefficient:** less than 7%

d. **Sensitivity:** not less than 200 copies of HBV DNA per 1 ml of blood plasma.

The claimed specifications are guaranteed when DNA extraction is performed with *PREP-NA* DNA/RNA Extraction Kit.

13 QUALITY CONTROL

"DNA-Technology, Research&Production" LLC declares that. the quality control procedures performed in accordance with ISO ISO 9001:2008 and ISO 13485:2003

14 KEY TO SYMBOLS

Caution

Manufacturer

Consult instructions for use

Negative control

Date of manufacture

Positive control

Expiration date

Cataloque number

In vitro diagnostic medical device

Sufficient for

Batch code

Temperature limitation

Version

Upper limit of temperature